CORRESPONDENCE

Prognostic Significance of Quantitative Viral Markers in Adult T-Cell Leukemia/Lymphoma

To the Editor:

Adult T-cell leukemia/lymphoma (ATL) is a T-cell non-Hodgkin's lymphoma frequently associated with a leukemic phase and is caused by human T-cell lymphotropic virus type I (HTLV-I).[1] Development of ATL is preceded by high HTLV-I antibody titers[2] and characterized by monoclonal integration of proviral DNA in mononuclear cells in peripheral blood and/or lymph nodes.[3] ATL has a broad clinical spectrum with several subtypes and corresponding differences in survival.[4] The acute and lymphoma subtype have a prognosis of less than 1 year, while chronic and smoldering have longer survival and frequently precede the more severe types. Because proviral DNA level (proviral load) and antibody titers are important diagnostic markers, we evaluated their utility as predictors of survival in ATL.

We analyzed 30 ATL patients (acute, lymphoma, chronic subtype) from the Non-Hodgkin's Lymphoma Registry at the University of the West Indies in Jamaica. All participants were enrolled in studies approved by Protocol and Human Subjects' Review Committees at the National Cancer Institute (Bethesda, MD) and the University of the West Indies (Kingston, Jamaica).

Serum samples were screened by whole-virus enzyme-linked immunoassay (EIA) (Dupont, Wilmington, DE) and confirmed by Western blot (Biotech, Rockville, MD). HTLV-I antibody titers were assayed by the end-point dilution method using an EIA (Genetic Systems, Seattle, WA, or Cambridge-Biotech, Rockville, MD) at fourfold dilutions. Quantitative proviral DNA levels were detected by a real-time automated polymerase chain reaction (PCR) method. Ten microliters of DNA was amplified for 45 cycles with AmpliTaq Gold polymerase using an ABI PRISM Sequence Detection System and TaqMan PCR Reagent [P/N N808-0230] (PE Applied Biosystems, Foster City, CA) in a 96-well format. The HTLV-I/II primers were from highly conserved sequences (GenBank National Center for Biotechnology Information, Bethesda, MD) from the *tax* gene [HTV-F5 (7358-7378) and HTV-R4 (7518-7499)]. Triplicate reactions were performed and unknown copy numbers were automatically calculated by interpolation from a plasmid control regression curve and reported as copy equivalents per 10^5 lymphocytes. The assay reliably detects at least 3 copies per 10^5 lymphocytes. Samples with undetectable virus were scored as 1 copy per 10^5 lymphocytes for calculations.

Kaplan-Meier life-table methods were used to estimate survival and 95% confidence intervals. The log-rank statistic was used to evaluate differences between survival curves. A dichotomized variable above or below the median value was used to compare survival by white blood cell count, proviral DNA, and antibody titer levels at diagnosis. Lymphocytosis was defined as lymphocyte count exceeding 4,000/μL. Each numeric value was $\log_{10}$ transformed and ultimately reported as arithmetic values. Kruskal-Wallis test was used to compare mean values between groups. Correlations were examined using Spearman's rank order statistic. All *P* values were two-sided.

Median survival was significantly shorter for acute (101 days, 95% confidence interval [CI] [54 to 124 days]) and lymphoma (83 days, 95% CI [63 to 314 days]) subtypes compared with the chronic subtype ($P = .009$) whose median survival could not be determined because 3 of 6 patients were still alive. Significant adverse clinical prognostic factors at diagnosis included hypercalcemia, hepatomegaly, splenomegaly, and B symptoms. Other factors that were not significant included white blood cell count and lymphocytosis. Proviral load and antibody titers were also not significant. Differences in proviral load between subtypes were significant ($P = .01$), with higher levels among acute and chronic subtypes compared with lymphoma subtype (Table 1). Interestingly, acute ATL patients had a higher white blood cell count compared with the chronic and lymphoma subtypes. The elevated white blood count among acute ATL cases did not correspond to the highest proviral load, although white blood cell count was significantly correlated with proviral DNA level in all patients ($R = .47$; $P = .009$). Chronic and acute ATL patients had similar median absolute lymphocyte counts (9,679 and 8,742 cells/μL, respectively) and about 50% of both subtypes had circulating abnormal lymphocytes. These data suggest that chronic ATL patients may have more detectable virus per cell. Antibody titer levels were similar between the subtypes ($P = .23$). However, a trend toward better survival, 471 days (95% CI 102 to 935) versus 83 days (95% CI 54 to 314), was observed for cases with titer levels above the median (1:29,765) at diagnosis.

Viral markers were not significant predictors of survival among ATL patients, although others have shown that the ability to detect proviral DNA declines in complete remission after therapy.[3] In this study, higher antibody titers at diagnosis appeared to be associated with longer survival. Chronic ATL patients with high antibody titer levels, reflective of a heightened immune response, either require no immediate therapy or respond well to various treatment approaches,[5] despite highest proviral load. The lymphoma subtype characterized by a low level of circulating malignant cells had the lowest proviral load but poorest survival. This may explain why these cases would fail to respond to anti–viral-containing regimens such as zidovudine and interferon-α[6,7] and other immunotherapeutic approaches.[5] To improve response rates among ATL patients, targeted, tumor-specific therapy that also enhances immunity is needed.

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Table 1. Proviral DNA and Antibody Titers Among ATL Patients by Clinical Subtype*

Subtype	N	Median White Blood Count × 10^3	Median Proviral DNA Levels (copies/10^5 lymphocytes)	Median Antibody Titers (reciprocal titer)
Acute	11	50.0	30,409	9,162
		(23.9, 178.7)†	(17,100, 72,946)	(4,406, 249,459)
Lymphoma	13	11.9	4,266	23,281
		(7.2, 12.2)	(3,177, 13,489)	(5,916, 68,234)
Chronic	6	17.1	81,283	97,949
		(14.8, 38.9)	(34,914, 144,877)	(59,020, 163,305)

*As defined in Shimoyama.[4]
†25th and 75th percentile values.

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Tyrosine Phosphorylation of Shc Proteins in Normal CD34$^+$ Progenitor Cells and Leukemic Cells

To the Editor:

After the Shc gene was identified, biological function studies have focused on the oncogenic potentials of Shc proteins, especially their role in the Ras-dependent mitogen-activated protein kinase activation.[1,2] Jucker et al[3] reported in *Blood* that Shc proteins are constitutively tyrosine-phosphorylated in primary acute myelogenous leukemia (AML) cells but not in primary cell cultures or normal tissues.[3,4] The presence of constitutively phosphorylated Shc proteins found in the AML peripheral blood (PB) cells, but not in normal PB cells, led to the suggestion that the Ras pathway may be constitutively activated in AML.[3] However, it should be noted that in these studies Shc phosphorylation was compared in PB cells of AML and PB cells of healthy donors. One potential problem with their conclusion is that AML cells are immature myeloid cells while normal PB cells are end-stage mature cells. Hence, the Shc phosphorylation difference between these two groups of cells might reflect difference in the level of cell maturation rather than a difference between leukemic and normal myeloid cells. To distinguish between Shc phosphorylation in these two groups of cells, one must compare Shc phosphorylation in AML cells and in normal bone marrow (BM) cells that are at a comparable level of maturation.

We evaluated Shc protein expression and phosphorylation in normal hematopoietic progenitor cells and leukemia hematopoietic cells. Five normal individuals and 10 AML patients participated in this study. Three leukemic cell lines (HL-60, K562, and KG-1) were also studied. BM and PB specimens were collected before any treatment from the 10 patients with newly diagnosed AML. BM aspirates were obtained from the five normal donors. Standard Ficoll density centrifugation was performed to collect mononuclear cells (MNCs). MNCs from BM specimens of two patients with AML and five normal donors were subjected to CD34 separation by MACS separation columns (Miltenyi Biotec Inc, Auburn, CA). Cell pellets of PB and BM were lysed in cell

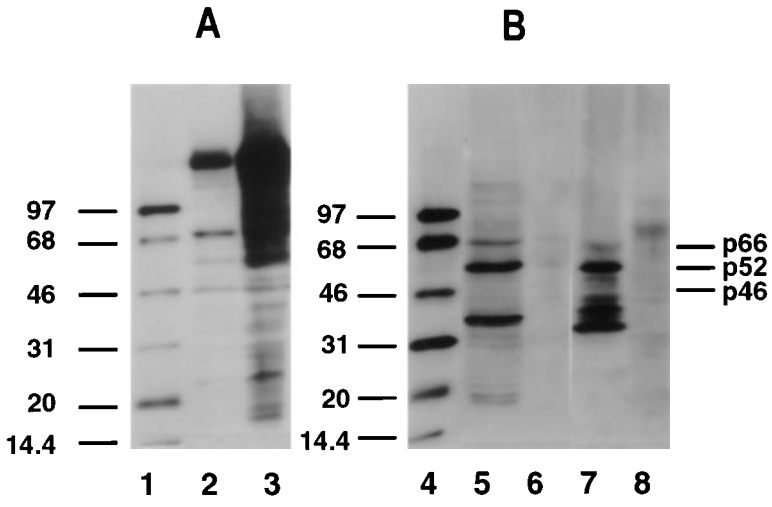

Fig 1. Tyrosine phosphorylation of Shc proteins in normal CD34$^+$ progenitor cells and leukemic CD34$^+$ cells. Total cell lysates (20 μg protein) were subjected to Western blot analysis. Phosphotyrosine was identified by antiphosphotyrosine antibody (4G10). Representative blots are shown. The positions of p66^{Shc}, p52^{Shc}, and p46^{Shc} are indicated on the right. Molecular-weight marker bands are indicated on the left. Lanes 1 and 4, enhanced chemiluminescence protein molecular-weight markers; lane 2, Nonstimulated A431 cell lysate; lane 3, epidermal growth factor–stimulated A431 cell lysate; lane 5, CD34$^+$ cells from normal BM; lane 6, CD34$^-$ cells from normal BM; lane 7, CD34$^+$ cells from AML patient BM; lane 8, CD34$^-$ cells from AML patient BM.